Appendix G

Notes on the Methodology for Evaluating Sediment Toxicity Tests

Results of sediment toxicity tests conducted around the United States were submitted with several databases for evaluation in the NSI. Additional processing of records was required for most of the data. Because test results were reported differently in each database, appropriate interpretation of the test results was sometimes confusing. This section explains how the toxicity test data were handled for the NSI evaluation with respect to issues related to sampling date, type of test, sample location identification, and results of control or reference tests conducted during the toxicity tests.

Sampling Date

Only those tests in the databases for which the sediment samples were obtained between January 1, 1980, and December 31, 1993, were evaluated. Tests before and after that period were eliminated.

Sample Location

Records were examined to determine whether the sampling station from which the sediment sample was collected had been identified by latitude and longitude coordinates. Samples that were not referenced to a specific location were not considered in this study. Tests from the Great Lakes Sediment Inventory (GLSI) database were not considered because sample locations were not appropriately identified. Sediment samples in the EPA Region 10/U.S. Army Corps of Engineers Seattle District's Sediment Inventory (SEACOE) from sampling stations located in British Columbia were also not considered in the analysis.

Type of Test

Data from seven databases (Table G-1) were reviewed to determine whether they had reported the results of sediment (solid-phase) and elutriate nonmicrobial toxicity tests in which the endpoint was mortality. Records pertaining to chronic toxicity tests, microbial toxicity tests, tests that were not conducted with sediment or elutriate, and tests in which the endpoint was not percent mortality (or percent survival, which could be converted to percent mortality) were excluded from further consideration.

Only the DMATS and GOM databases clearly reported the phase (solid, elutriate, particulate) of sediment sample used in the bioassays conducted; ODES provided this information for some of the tests. If the phase was not indicated, this information was obtained or best professional judgment was used to identify the phase used in the tests. For some tests, comparison of species with those used in standard EPA test protocols or with species used in other sediment toxicity tests in the databases permitted assignment of phase with certainty. Other species might be used in sediment-, elutriate-, and particulate-phase tests, and the phase was assigned with uncertainty. Table G-2 presents a list of species used in toxicity tests whose results are included in the NSI. Table G-2 also presents the type of toxicity test for which each species is generally used (i.e., liquid-phase, elutriate-phase, suspended particulate-phase, sediment/solid-phase). The data presented in Table G-2 are the basis for determining whether the toxicity test of concern was conducted using the solid or elutriate phase. A "Y" entered in Table G-2 indicates that the phase was given with the test results; an "E" indicates that the phase was estimated using best professional judgment based on the species used in the toxicity test.

Table G-1. Toxicity Test Database Characteristics

Database	Sample Locations Identified by Lat/Long	Type of Test	Laboratory Control Tests	Reference Sediment Tests	Comments
U.S. Army Corps of Engineers, Dredged Material Tracking System (DMATS)	Yes, all 74	Solid and Elutriate (identified in database)	Replicate control test results provided	Replicate reference sediments tested with each batch of sediment samples	Used means of reference sediment replicates in the evaluation (contact: Alan Ota, EPA Region 9)
EPA's Environmental Monitoring and Assessment Program, Louisianian Province (EMAP-LA)	Yes, all 259	Solid Phase (not identified in database, provided)	Not provided in D3 database, provided on request	No	Sediment sample test results were calculated from the additional data provided (contact: Kevin Summers, EPA/ERLGB)
EPA's Environmental Monitoring and Assessment Program, Virginian Province (EMAP-VA)	Yes, all 179	Solid and Elutriate (not identified in database, provided)	Not provided in D3 database, provided on request	No	Sediment sample test results were calculated from the additional data provided (contact: Daryl Keith, EPA/ ERLN)
Gulf of Mexico Program's Contaminated Sediment Inventory (GOM)	Yes, all 42	Solid Phase (identified in database)	ERL-N: Yes USACE: No GCRL: No, provided on request	ERL-N: Yes USACE: Yes GCRL: No	Long Island Sound reference sediment was used to generate control data for tests done by ERL-N (contacts: Phil Crocker, EPA; John Scott, SAIC) and control data obtained for GCRL (contact: Julia Lyle, GCRL); for USACE tests used mean of the reference test results as control
EPA's Great Lakes Sediment Inventory (GLSI)	No	Not identified in database	Not provided in database	No?	Sample location IDs and control test reults were not provided; therefore, these data were not evaluated for the NSI (contact: Bob Hoke, SAIC)
EPA's Ocean Data Evaluation System (ODES)	Only 18 out of 68	Solid Phase (not identified in database)	Yes	No	Used controls (contact: Tad Deschler, Tetra Tech)
EPA's Region 10/U.S. Army Corps of Engineers Seattle District's Sediment Inventory (SEACOE)	Only 18 out of 68	Solid Phase (not identified in database)	Yes, some had to be provided on request	Yes	Used controls (contact: Roberts Feins, Environmental Information Consultants; John Armstrong, EPA Region 10; and Gary Braun, Tetra Tech, for Puget Sound Estuary Program Reports, 1988)

Table G-2. Test Species Used in Sediment Bioassay Test Results Included in the NSI

Species Code	Species Name	*Type of Toxicity Test*							
		Liquid (L)	Elutriate (E)	Particulate (P)	Solid (S)	C (L most Common) (L or E)	D (L,E, or P)	A (L,E,P,or S)	Unknown
80509070600									E
615301010900	*Acanthomysis costata*		Y		Y				
615301010400	*Acanthomysis macropsis*	Y	Y		Y				
615301010700	*Acanthomysis sculpta spp.*	Y				E			
611829010000	*Acartia spp.*	Y	Y						
616902010800	*Ampelisca abdita*				Y,E				
616800000000	*Amphipods*				Y				
610401010100	*Artemia salina*	Y	Y						
616302070900	*Asellus intermedius*				E				
650508331700	*Chironomus riparius*				E				
650508330100	*Chironomus tentans*				E				
885703010200	*Citharichthys stigmaeus*	Y			Y				
616915021500	*Corophium spinicorne spp.*				Y,E				
617922010000	*Crangon spp.*	Y	Y		Y				
551002010100	*Crassostrea gigas*		Y		Y		E		
551002010200	*Crassostrea virginica*				Y				
880404010100	*Cyprinodon variegatus*	Y		Y					
610902010900	*Daphnia magna*					E			
610902010100	*Daphnia pulex*					E			
815501010100	*Dendraster excentricus*					E			

Table G-2. (Continued)

Species Name	Species Name	*Type of Toxicity Test*							
		Liquid (L)	Elutriate (E)	Particulate (P)	Solid (S)	C (L most Common) (L or E)	D (L,E, or P)	A (L,E,P,or S)	Unknown
880404020700	*Fundulus grandis*	Y		Y					
881801010100	*Gasterosteus aculeatus*					E			
616915090200	*Grandidierella japonica*				Y				
622003030700	*Hexagenia limbata*				E				
615301010700	*Holmesimysis sculpta*	Y	Y		Y	E			
616923040100	*Hyallella azteca*				E				
500501010300	*Lumbriculus variegatus*				E				
814802010200	*Lytechinus pictus*	Y	Y						
551531011600	*Macoma balthica*				E				
551531011400	*Macoma nasuta*		Y		Y,E				
551531010000	*Macoma* spp.				E				
615303140600	*Metamysidopsis elongata*	Y	Y		Y				
651530100000	*Mysid shrimp*	Y		Y	Y				
615301210200	*Mysidopsis bahia*			Y	Y				
550701010100	*Mytilus edulis*	Y	Y					E	
500124030500	*Neanthes arenaceodentata*				Y,E				
500124030000	*Neanthes* spp.				E				
500125011900	*Nephtys caecoides*				Y,E				
500124030200	*Nereis virens*				Y				
551706040100	*Panopea generosa*				E				

Table G-2. (Continued)

Species Code	Species Name	*Type of Toxicity Test*							
		Liquid (L)	Elutriate (E)	Particulate (P)	Solid (S)	C (L most Common) (L or E)	D (L,E, or P)	A (L,E,P,or S)	Unknown
	Paratanytarsus parthogenetic				E				
617701010200	*Penaeus duorarum*				Y				
MICROTOX	*Photobacterium phosphoreum*					E			
877601160200	*Pimephales promeles*	Y				E			
551547070100	*Protothaca staminea*				Y				
616942150400	*Rhepoxynius abronius*				Y,E				
080309070600	*Selenastrum capricornutum*								E
814903020400	*Strongylocentrotus purpuratus*	Y	Y			E			
611910030100	*Tigriopus californicus*								E

Only DMATS contained elutriate test results in addition to sediment test results; all other tests evaluated were sediment (solid- phase) test results.

Test Controls

Toxicity data were screened to determine whether control data were reported. Sediment toxicity test laboratory or performance controls are usually clean sand or sediment run under the same conditions in which the same test organisms are exposed at the same time as those exposed to the sediment samples tested. Controls are used to determine whether observed mortality might be the result of the quality of test organisms used or other factors, and not the result of exposure to possible toxics in the sediment samples.

The databases were screened to locate control test data for each sediment sample tested. The GLSI database did not contain any control test data; because of this, as well as the lack of station-identifying coordinates, the GLSI database was eliminated from evaluation for the NSI. For the other databases, control test results were matched to the sediment test results and were treated as follows:

- Multiple control and reference sample test results were reported for each sediment tested in the DMATS database. These were determined to be replicate test results. Because the sediment samples tested in DMATS were usually fine-grained and the laboratory performance controls were sand, the reference sediment samples were used as "controls" to evaluate toxicity of sediment samples. The percent mortality for the reference replicates were averaged for each reference site to obtain the mean percent mortality for the reference sediment for comparison with the sediment sample test result.

- The D3 version of both the EMAP-LA and EMAP-VA databases contained control-corrected results for the sediment samples tested. The control-corrected results were obtained using the following equation:

$$\frac{\textit{percent survival of organisms in sediment sample test}}{\textit{percent survival of organisms in control test percent survival}} = \textit{control-corrected percent survival}$$

- EMAP-LA provided a revised database on request that contained the percent survival of the controls. The sediment sample test results were calculated according to this equation:

$$\textit{percent survival of organisms in sediment sample test} = \frac{\textit{control-corrected percent survival X percent survival of organisms in control test}}{100}$$

- EMAP-VA provided a revised database on request that contained the mean percent mortality of controls and the mean percent mortality of the sediment sample tests for each station, as well as the control-corrected percent survival.

- The GOM database reported control test results for tests conducted by EPA's Environmental Research Laboratory in Narragansett. A low-salinity control test performed at the same time was not used in the evaluation. The single reference sediment sample was treated as a sediment toxicity test result. No control tests were available from the USACE data set within this database; the mean of reference sediment toxicity test results was used as the "control" for these test data. No control test results were found in the GOM database for the GCRL data set. Total percent mortality of pooled control test replicates were provided by Julia Lytle of GCRL and entered into the database for the NSI analysis.

- The ODES database reported single-value control results for the ARSR and OSE data sets. (Whether these were means of replicate tests is unknown.) One sediment test result in ARSR was matched to two different control test results; however, the one control test result that was not matched elsewhere in the data set was eliminated for the analysis.

- The SEACOE database contained single-value control test results for the ALCTRAZ data set and several series of control test results for other data sets (e.g., EVCHEM and EBCHEM). Information on the correct control series was obtained, and the proper control test results were evaluated in the computer program. Means were calculated for replicates in the series and used to evaluate the sediment sample test results.

Results of control tests reported as "percent survival" were converted to "percent mortality" by the following calculations:

percent mortality = 100 - percent survival

percent mortality = number of surviving organisms/total number of organisms in test

Sometimes entries in databases reversed "mortality" and "survival" (e.g., PSE data set in the ODES database). Any questions concerning the designation were checked and corrected if necessary. If replicate sediment toxicity test results were provided for a sampling site in the database, a mean was calculated and compared to the mean control mortality. (Some databases provided only the means, e.g., EMAP-LA, EMAP-VA.) For the purpose of the NSI evaluation, if the control had greater than 20 percent mortality (less than 80 percent survival), that test was excluded from further consideration.

Reference Sediment Stations

Some data sets included data for reference sediments that were run simultaneously with the control and sediment samples. Reference sediment is sediment collected from a field site that is appreciably free of toxic chemical contaminants and has grain size, total organic carbon, sulfide and ammonia levels, and other characteristics similar to the sediment samples to be tested for toxicity. Because reference sediments should match the characteristics of the sediment samples more closely than the sand or sediment used for the laboratory (performance) control, they should provide information on the appropriateness of using a particular test organism since the suitability and survival of different species can be affected by these other physical and chemical characteristics of the sediment.

- As noted previously, DMATS provided several reference sediment samples for each toxicity test, along with control test results. The number of such reference sediment samples varied for different test dates, and these sediment samples were determined to represent replicates. The average percent mortality was determined from each set of replicates and this was used as a "control" to evaluate the toxicity of sediment samples in this database. If percent mortality of the mean reference test result exceeded 20 percent, the sediment toxicity tests that were run with that reference sediment were not used in the evaluation.

- Reference sediment test results were not identified in the EMAP-LA, EMAP-VA, or ODES databases.

- In the GOM database, a reference sediment test was run in tests conducted by EPA's Environmental Research Laboratory in Narragansett. This single reference sediment sample was treated as a sediment toxicity test result. Reference sediment tests in the USACE data set were averaged and used as the control for analysis since other control test data were not provided in the data set.

- Reference sediment toxicity test results in the SEACOE database were treated as a sample site.

Because reference toxicity test results were not available for all of the sediment toxicity tests, reference sediment sample test results were not used as "controls" in the evaluation of sediment toxicity test data in the NSI, with the exception of the DMATS data and the USACE data in the GOM database. The remaining reference sediment test results were compared with the control results to determine whether significant toxicity was indicated at that field site; i.e., they were treated like a sediment toxicity test result (see below).

It should be noted, however, that careful examination of such reference test results could improve the interpretation of sediment toxicity tests; i.e., they might indicate that test organisms were adversely affected by sediment characteristics, not by toxic chemicals. Thus, the classification of some sites using the sediment toxicity tests might

be inappropriate because the control test result did not adequately explain the result, based on the test organism's health or sensitivity to test conditions.

Test Results

For the NSI evaluation protocol for sediment toxicity test data, significant toxicity was indicated if there was a difference of 20 percent survival from control survival (e.g., if control survival was 100 percent and 80 percent or less of the test organisms survived, or if control survival was 80 percent and 60 percent or less of the test organisms survived, significant toxicity was indicated). Although a number of different test species and protocols were used in the tests evaluated, this threshold provides a preliminary indication of sediment toxicity for classifying sampling stations for the NSI.